Enhancers of innate and adaptive immunity combine with membrane bound IL15 to increase the efficacy of tumor infiltrating lymphocyte (TIL) therapy for tumors with immunosuppressive microenvironments

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Abstract 369

Background

The clinical impact of tumor infiltrating lymphocytes (TIL) cell products is currently limited by suboptimal persistence and potency, as well as the need for high-dose adjuvant IL-2 treatment, which is associated with severe toxicities. Thus, we engineered an IL-2-independent TIL product, based on regulated expression of interleukin 15 (cytoTIL15™ cells), which has shown anti-tumor efficacy and persistence in human melanoma PDX models. Since the immuno-suppressive tumor microenvironment (TME) hinders cell therapies, we hypothesized that combining pleotropic cytokines of the interferon (IFN), IL-1, or TNF families with IL-15 would further enhance antitumor activity and that our cytoDRiVE® platform would allow pharmacologic control of these potent immune mediators. We tested constitutive and regulated combinations of a representative member of these cytokines with IL-15 in human TIL for *in vitro* polyfunctionality and *in vivo* antigen-independent persistence. We also engineered mouse pmel-TCR cells with cytokine combinations for evaluation in the syngeneic B16 melanoma model.

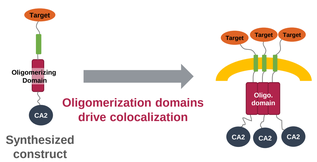
The Obsidian cytoDRiVE® platform

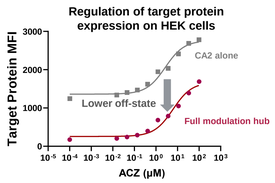
Obsidian's cytoDRiVE® platform coupled with modulation hubs can be used to control protein expression at low off-states, acting as a titratable and reversible rheostat for on demand activity

DRD: Carbonic Anhydrase 2 (CA2)
Ligands: Acetazolamide (ACZ), Celecoxib

- Drug responsive domains (DRDs)
  - Off-state = in the absence of ligand, the DRD is unfolded and degraded by the proteasome along with the target
  - On-state = in the presence of ACZ the DRD is stabilized allowing for target protein expression and function
- Carbonic Anhydrase DRD is fully human
- The stabilizing small molecule ligand, Acetazolamide (ACZ) is
  - Orally bioavailable
  - FDA approved

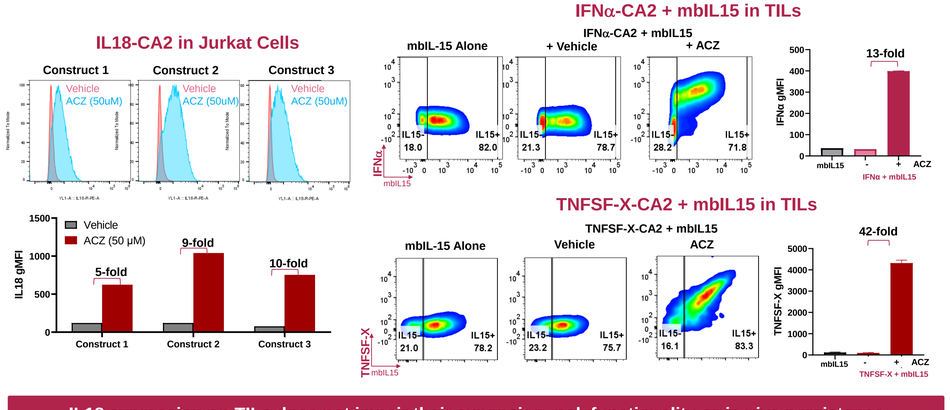
DRD Modulation Hubs: Adding Oligomerization Domains Improves Regulation





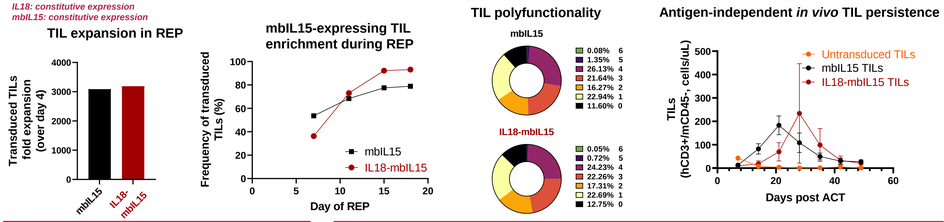
Results

Expression of enhancers of innate and adaptive immunity is efficiently regulated in Jurkat cells and engineered Tumor-Infiltrating Lymphocytes (TILs)



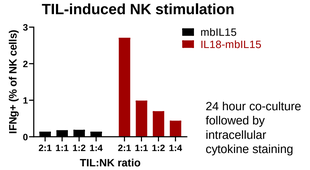
IL18 expression on TILs does not impair their expansion, polyfunctionality or *in vivo* persistence

IL18: constitutive expression
mbIL15: constitutive expression



IL18-mbIL15-expressing TILs induce IFNγ production in NK cells

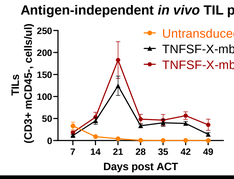
IL18: constitutive expression
mbIL15: constitutive expression



24 hour co-culture followed by intracellular cytokine staining

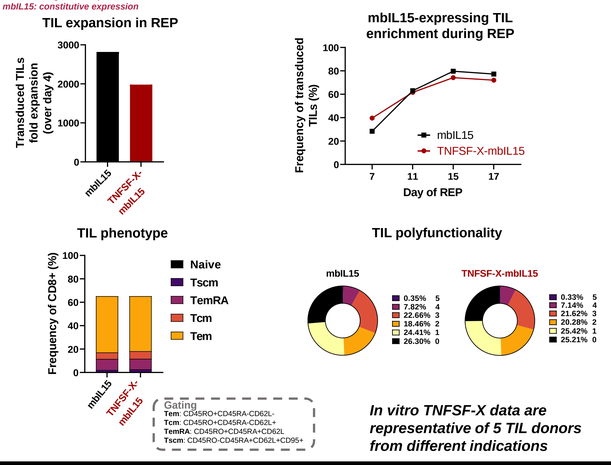
ACZ-induced TNFSF-X expression on TILs does not alter their *in vivo* persistence

TNFSF-X: regulated expression
mbIL15: constitutive expression



TILs transduced with constitutive mbIL15 and regulated TNFSF-X show similar expansion, phenotype and polyfunctionality compared to mbIL15-TILs

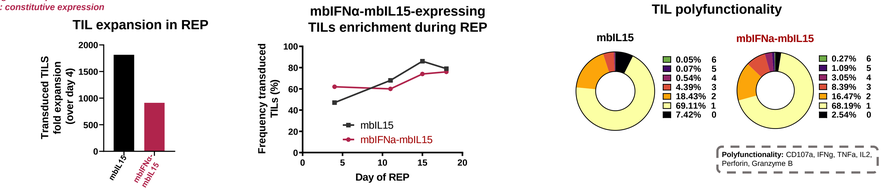
TNFSF-X: regulated expression
mbIL15: constitutive expression



In vitro TNFSF-X data are representative of 5 TIL donors from different indications

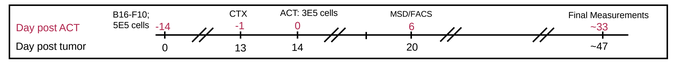
TILs transduced with constitutive mbIL15 and regulated IFNα expand with increased polyfunctionality

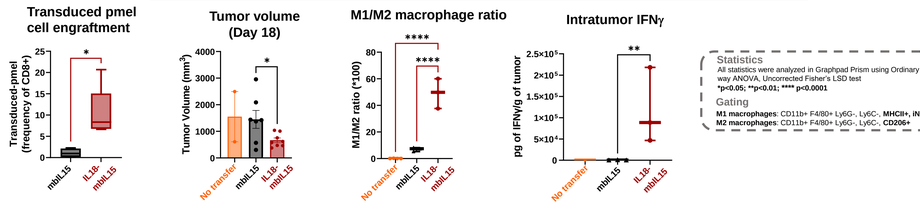
IFNα: regulated expression
mbIL15: constitutive expression



Mice engrafted with CD8 pmel TCR-transgenic T cells expressing IL18 and IL15 show improved control of B16 tumors and a pro-inflammatory tumor microenvironment

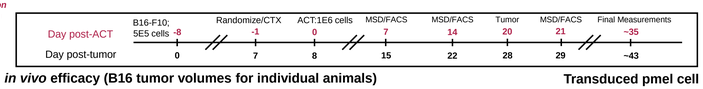
IL18: constitutive expression
mbIL15: constitutive expression

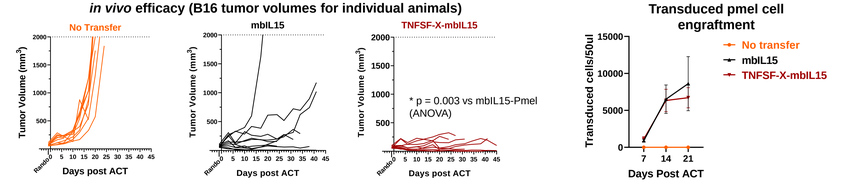




Mice engrafted with CD8 pmel TCR-transgenic T cells expressing TNFSF-X and mbIL15 show significant durable B16 tumor growth inhibition

TNFSF-X: constitutive expression
mbIL15: constitutive expression





Conclusions

- Obsidian's cytoDRiVE® platform allows pharmacologic control of potent immune mediators including IL18, IFNα and TNFSF-X
- cytoTIL15™ cells, Obsidian's TIL expressing mbIL-15 that expand and persist without IL-2, can be further engineered to express regulated enhancers of innate and adaptive immunity that can modify the tumor microenvironment to enhance TIL potency for solid tumors marked by an immunosuppressive TME

For questions, please reach out to Carmela Passaro (cpassaro@obsidiantx.com)

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